6/8/16

Wednesday, June 22, 2016
12:30 PM

Digested registry parts BBa_K206000 ("14A"), BBa_K206001 ("14C"), and BBa_I732094 ("1P").

14A:
7.12 uL DNA
8 uL CutSmart
2 uL EcoRI
2 uL SpeI

14C:
8.76 uL DNA
8 uL CutSmart
2 uL EcoRI
2 uL SpeI

1P:
7.00 uL DNA
8 uL CutSmart
2 uL EcoRI
2 uL XbaI

Incubate 1 hour at 37C.
Heat kill 20 minutes 80C.

Add 4 uL SAP to 1P, incubate 30 minutes 37C.
Heat kill 20 minutes 80C.

Loaded 14A, 14C, 1P into an .8% gel at 100 V for 40 minutes.

Strange results from gel, so tested enzymes against Michelle's. Repeated protocol above with ¼ the volume. Two sets of digests, one with iGEM enzymes, one with Michelle's.

6/9/16

Monday, June 27, 2016
11:16 AM

Ran enzyme test from yesterday.

Order of loading (left to right):
1P (Michelle)
14C (Michelle)
14A (Michelle)

1P (iGEM)
14C(iGEM)
14A (iGEM)

No ladder as it was used up. Gel saved to computer in iGEM folder.

6/14/16

Monday, June 27, 2016
11:26 AM

Another digest test. Part BBa_K1399007 ("7N") digested with SpeI and EcoR1 using standard digest protocol and ran in gel (1% agarose, 120V, 25 min) against undigested plasmid.

Clear bands in digested sample indicate our enzymes are working, and that problems with previous day's gels are due to incorrect protocol or gel conditions.

6/16/16

Monday, June 27, 2016
11:29 AM

Repeated protocol from 6/8/16.

6/20/16

Monday, June 27, 2016
11:29 AM

Ran digests from 6/6/16 on 0.7% gel, 100V, 25 minutes. Unable to visualize clear bands for gel extraction.

6/21/16

Monday, June 27, 2016
11:30 AM

Repeated protocol from 6/8/16.

7/8/2016

Friday, July 8, 2016
1:54 PM

Resuspended GFP Testing Construct V1 Gblock in 100 uL ddH20 (final concentration: 10 ng/uL)
Diluted gBlock 1:10 to make 10 uL of 1 ng/uL gBlock.
Ran PCR using cycle "GFP00short" (only 10 cycles) and placed 1 sample tube in column 7 of gradient, one in column 9. Primers 20 (forward) and 21 (reverse), used 1 ng of gBlock template, all else according to Q5 Mastermix instructions.
Diagnostic gel showed only bands of size >100 which I'm assuming were primers. Saved the image but threw away the PCR product tubes.

7/27/16

Wednesday, July 27, 2016
11:38 AM

Spun down digested and PCR purified samples from yesterday (pSB1C3 backbone and GFP gBlock) and resuspended in 10 ul ddH20 to increase concentration.

GFP concentration was still low (4 ng/ul), so repeated digestion protocol from yesterday on GFP gBlock.

7/28/16

Thursday, July 28, 2016
2:12 PM

Prepared competent dh5-alpha cells according to the following protocol:

1. Prepare the following:
   - LB
   - 0.1 M CaCl2 (keep cold)
   - 0.1 M CaCl2-15% glycerol (keep cold)
   - 1.5 ml eppendorf tubes (keep cold)
2. Grow 50 uL of old comp cells in a 5 ml preculture overnight in LB
3. Make a 1% inoculation to 50 ml LB (in 250 ml flask)
4. Place in shaker at 37°C until OD 0.5 (takes about 1.5 hours)
5. Set centrifuge to 4 degrees C.
6. Transfer 50 ml culture to 50 ml falcon tube
7. Centrifuge for 10 min at 3000 rpm at 4°C
8. Remove supernatant as much as possible and resuspend cells in 5 ml 0.1 M CaCl2 using pipette tips
9. Rest the tube for 30 min on ice
10. Repeat 7-9 two more times, and then proceed to 11
11. Centrifuge with same settings as 7, and then completely remove supernatant
12. Resuspend cells in 2.5 ml of 0.1 M CaCl2-15% glycerol
13. Aliquot 50 ul to sterile 1.5 ml tubes and keep in -80°C

Stored in the far left freezer, bottom shelf.

8/2/16

Thursday, July 28, 2016
2:13 PM

Removed puc19 plates from last night's transformation (to test comp cell efficiency). >100 colonies; comp cells are good!

Attempted another PCR of Gblock using "GFP00short" program. Still poor results; Todd suggested increasing the number of cycles from 10 to 25.

8/8/12

Monday, August 8, 2016
10:08 AM

Today's plan is to attempt two different types of assembly for the GFP part in parallel.

Gibson Assembly:

PCR backbone with primers IGEM26 and IGEM27
Annealing temperature set to 63C. One 50 uL tube was prepared according to Q5 Mastermix instructions and set for 30 cycles.
Add with Gblock straight from the tube into the reaction according to the standard protocol

8/9/16

Wednesday, August 10, 2016
3:04 PM

Finally got a good PCR of the Gblock! Used BufferG and Q5 polymerase at standard settings, 63C annealling temperature for 30 cycles. Primer dimers and smear still evident on the gel, but there was a sharp enough band to cut and purify using the Qiagen gel purification kit with Mariam. Afterward, Mariam used that product for her Gibson reaction to assemble it into the pSB1C3 backbone.

Wednesday, August 10, 2016
3:05 PM

8/10/16

Wednesday, August 10, 2016
3:05 PM

Checked plates from last night's transformations. The Gibson was unsuccessful, but there were two colonies on the plate from Augustine's ligation. I inoculated both of them overnight in 5 ml LB + 34 ug/ml (8.75 ul of the 20 mg/ml stock solution) chloramphenical.

All 5 of the interlab study kit transformations also yielded at least a handful of colonies per plate. I parafilmed all plates (except the unsuccessful Gibson transformation, which I threw out) and set in the 4C fridge for later.

8/17/16

Monday, August 22, 2016
10:24 AM

With Augustine, completed Day 2 of the Interlab Study according to the protocol.

Inoculated the new GFP part (Ligation and Gibson versions) into LB + chloramphenicol overnight.

8/18/16

Monday, August 22, 2016
10:24 AM

With Augustine, completed Day 3 of the Interlab Study according to the protocol. All data was saved to excel spreadsheet on the lab computer for future analysis.

Completed miniprep of overnight inoculations from 8/17.

8/22/16

Monday, August 22, 2016
10:26 AM

8/24/16

Wednesday, August 24, 2016
4:08 PM

Removed overnight cultures from incubator. OD of the two GFP parts which had been induced was very low. Realized induction should not have happened during inoculation, but instead during exponential growth.

At 10:30, took 50 ul of each part (negative control, Gibson GFP, and ligation GFP) and added them to 5 ml of LB + chloramphenicol. 2 replicates of each.

At 3:45, took samples from each tube, put in well plate on ice, and induced one set of replicates with 20 ng/ml of tetracycline before returning to 37C shaker.

4:45: took additional samples of each culture and added to well plate. Returned to 37C incubator.

5:45: took additional samples of each culture and added to well plate. Placed in 30C incubator overnight.

8/25/16

Friday, September 9, 2016
2:51 PM

Could find no significant fluorescence from any of the induced parts when measured in plate reader. Sent New Ligation and New Gibson GFP parts in for sequencing at the Core Facility.

8/29/16

Friday, September 9, 2016
2:54 PM

Sequencing results were negative. ¾ reactions failed, and the last showed significant deletions. See attached sequencing report.

New GFP cassette will be ordered through IDT, this time in the provided IDT cloning vector to minimize the introduction of mutations.

9/9/16

Friday, September 9, 2016
2:54 PM

Ran the following PCR reactions:

4202 Sub
2.5 ul of 10 uM IGEM30 primer
2.5 ul of 10 uM IGEM31 primer
2 ul of e coli MG1655 genomic DNA
25 ul of NEB Q5 Mastermix
18 ul H20

98C for 30s
------cycle 30 times-----
98C for 10s
61C for 30s
72C for 30s

72C for 2 mins
Hold at 4C

3321 Sub
2.5 ul of 10 uM IGEM32 primer
2.5 ul of 10 uM IGEM33 primer
2 ul of e coli MG1655 genomic DNA
12.5 ul of NEB Q5 Mastermix
30.5 ul H20

98C for 30s
------cycle 30 times-----
98C for 10s
64C for 30s
72C for 30s

72C for 2 mins
Hold at 4C

1304 Sub
2.5 ul of 10 uM IGEM34 primer
2.5 ul of 10 uM IGEM35 primer
2 ul of e coli MG1655 genomic DNA
12.5 ul of NEB Q5 Mastermix
30.5 ul H20

98C for 30s
------cycle 30 times-----
98C for 10s
58C for 30s
72C for 30s

72C for 2 mins
Hold at 4C

Ran 5 uL of each on 1% agarose gel stained with Midori Green at V for 27 minutes. Imaged in GelDoc.

10/1/16

Sunday, October 2, 2016
1:11 PM

GFP Reporter V2 was received from IDT.

Reporter was resuspended in IDTE and transformed into competent DH5-alpha using the standard transformation protocol.

10/2/2016

Sunday, October 2, 2016
1:12 PM

Inoculated single colonies from last night's GFP V2 construct transformation into two overnight cultures of LB + ampicillin.

10/3/16

Tuesday, October 18, 2016
11:15 PM

Using standard digestion protocol, digest GFP-V3 part with Pst-I and Spe-I.
Gel purify digestion product using Qiagen kit.

10/4/16

Tuesday, October 18, 2016
11:18 PM

Using linearized backbone prepared by Mariam, ligate 50 ng of digested GFP-V3 part to pSB1C3 backbone. Used T4 DNA Ligase from NEB and followed standard protocol. 20 minute ligation time.

Transformed 3 ul of ligation product into DH5-alpha competent cells using the standard protocol and let incubate overnight.

10/5/16

Tuesday, October 18, 2016
11:21 PM

Inoculate three colonies from last night's GFP-V3 ligation into pSB1C3 in 5 mL LB + CM and set in 37C shaking incubator overnight.

10/6/16

Tuesday, October 18, 2016
11:22 PM

Extracted DNA from the 3 overnight colonies from last night using Qiagen miniprep kit. Elute in 30 uL NF-H20; otherwise followed protocol exactly.

10/7/16

Tuesday, October 18, 2016
11:25 PM

Digested 500 ng of each of the three colonies' GFP-V3 parts which were prepared yesterday. Digested using Eco-RI and Pst-I according to standard protocol and ran on gel for 27 minutes at 120V. Imaged gel and saved image on lab computer. Colony 3 showed banding (albeit faint) at the correct locations.

10/8/16

Tuesday, October 18, 2016
11:28 PM

Attempted Golden Gate assembly using the Colony 3 GFP part as the vector and each of the gel purified promoters purified by Mariam as the insert. Followed the Golden Gate protocol included with the NEB kit with no deviations.

Transformed 3 ul of the Golden Gate reaction product into DH5-alpha competent cells using the standard protocol and placed in the 37C incubator overnight.

10/10/16

Tuesday, October 18, 2016
11:34 PM

Checked colonies from Golden Gate plates from two days ago. Negative control contained as many colonies as the experimental plates, raising some concerns. Nevertheless, we grew five colonies from each plate overnight in 5 ml LB + CM.

10/11/16

Tuesday, October 18, 2016
11:36 PM

Miniprepped the overnight colonies from Golden Gate transformations from the last two days. Tested the Golden Gate assembly of promoter 3321 by digesting with EcoRI and SacI, which should have cleaved the plasmid in the middle of the inserted promoter. Ran the digestion product on 1% agarose gel at 120V for 27 minutes and imaged the result. Banding was identical between negative and positive controls, with no observable digestion. Concluded that Golden Gate assembly did not work as intended.

10/13/16

Tuesday, October 18, 2016
11:41 PM

Pelleted the overnight cultures prepared yesterday by centrifugation and stored in the –20C freezer until they could be miniprepped.

10/15/16

Tuesday, October 18, 2016
11:45 PM

Digested the miniprepped plasmid from the cells pelleted on 10/13. Digested again with EcoR1 and PstI according to the standard protocol and ran on gel at 120V for 27 minutes. Gel image was saved to lab computer. As before, no banding was identified compared to the control plasmid, indicating a negative result.

10/17/18

Tuesday, October 18, 2016
11:48 PM

Completed PCR of linearized pSB1C3 backbone according to the protocol found on the iGEM registry.